

# Plastic Properties of Higher Fatty Acid Derivatives of Proteins

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Some plastic properties of higher fatty acid derivatives of casein and of other proteins are reported. Data on the water absorption and tensile and flexural strengths of molded test specimens are compared with similar data for casein hardened with formaldehyde. Measurements of the flow properties of the molding powders were also made. The acylated casein derivatives flow well in small positive-type molds, and the molded pieces do not require further treatment with formaldehyde to yield finished articles.

A PREVIOUS paper from this laboratory demonstrated that the affinity of casein for water could be curtailed by acylating the protein with lower fatty acid anhydrides (7). Test specimens molded from the products, however, tended to be brittle and weak. In attempting to overcome this difficulty and, at the same time, to retain the characteristic of reduced water uptake, a series of new fatty acid derivatives of proteins was prepared and characterized. It is the purpose of this report to appraise these new products as thermoplastic molding powders with respect to the water absorption and tensile and flexural strength of molded specimens and to the flow properties of some of the powders.

The procedure for the preparation of the derivatives has been described in detail (6). It may be summarized briefly as follows: The protein is dissolved in aqueous alkali and treated with fatty acid chloride and sufficient alkali to maintain the pH at the desired level. The reaction mixture is then acidified to precipitate the derivative and excess fatty acid; the precipitate is filtered, washed thoroughly with water, dried, and extracted with ether.

The products derived from casein were white or yellowish powders, the color depending on that of the acid chloride used in the preparation. In turn, molded specimens were colored light yellow to dark yellow, depending on the color of the powder. All the molded objects were translucent, and many were almost transparent. The most highly acylated derivatives made the clearest molded pieces.

Before molding, most of the modified proteins were hardened with formaldehyde (4, 7). The treatment was carried out in either of two ways, without noticeable differences in the final products. The air-dried, ether-extracted derivative was suspended in three times its weight of 10% formaldehyde solution, and the suspension was stored at room temperature for 3 or 4 days. The product was filtered, washed thoroughly with water, and dried in the air. Alternatively, the filter cake of acid-precipitated derivative and fatty acid, while still moist from the water wash, as broken up in enough water to give a thick suspension, and formalin was added to adjust the formaldehyde concentration of the solution to roughly 10%. This suspension was also kept at room temperature for 3 or 4 days. The precipitate was then filtered, washed with water, dried, and extracted with ether. The

pH values of the formaldehyde solutions were determined but not controlled; they were within the range 3.5 to 5.0.

Test disks for the determination of water absorption were molded in the manner described previously (7), water again being used as plasticizer. However, it was not possible to utilize identical conditions in the molding operations for all the products in this series. A maximum molding temperature of 113–117° C. was employed uniformly, but, with different derivatives, pressures ranging from 2000 to 5000 pounds per square inch and water contents ranging from 6 to 12% were used. Selection of conditions was dependent upon the flow characteristics of the individual powders. For basic comparisons of the water uptake of disks molded under different conditions, data were obtained by the method of Mellon (8), whereby water contents at approximate saturation are obtained. Water absorption values at 24 hours were also determined by the A.S.T.M. procedure (1) to permit comparison of the materials with commercial plastics.

Test bars (0.5 × 0.5 × 5 inches) for determinations of tensile and flexural strengths were molded under the conditions described. For the tensile strength measurements the bars were cut in half. Each piece (0.5 × 0.5 × 2.5 inches) was turned down in a lathe with a sharp cutting tool pivoted on an arc of 3-inch radius; the dimensions of the final test specimens were a length of 2.5 inches and a minimum diameter of 0.200 ± 0.004 inch. The machined specimens were conditioned for at least a week at 77 ± 2° F. and a relative humidity of 50 ± 2%. On removal from the conditioning atmosphere they were tested immediately in a room held at 70 ± 2° F. and 65 ± 2% relative humidity, on a modified J-2 Scott tester of 440 pounds capacity equipped with self-aligning grips especially designed for the machined specimens. The mean rate of loading was 40.6 pounds per square inch per second. At least three, and in most cases six, specimens of each material were broken. The tensile strength figures in the tables are average values. That the values obtained by this method of testing are reliable was recently verified by comparative measurements, on a Tinius-Olsen Plastiversal machine, of the tensile strength of similarly machined pieces. Further details of this testing procedure will be published elsewhere.

Flexural strengths were determined directly on the molded test bars under the same conditions of temperature and humidity described above and after a similar conditioning period. The testing machine used was that described by Brother, Suttle, and McKinney (5). The flexural strength figures (modulus of rupture) listed in the tables are averages of two or three individual tests. The procedure employed thus deviates from the A.S.T.M. method (2) with respect to the conditioning period and the number of specimens tested.

Measurements of flow properties were made, in general, according to the A.S.T.M. procedure (3, Method B). Preformed tablets were made in a tablet press from powders which contained 12 and 8.5% water. The tablets were not conditioned because water was used as a plasticizer. They were tested immediately after being preformed so that their actual water content would not differ

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TABLE I. WATER ABSORPTION OF PALMITOYL CASEINS

Sample No.	Palmitoyl Content, %	Water Absorption of Disks, %		
		Unhardened	Hardened with Formaldehyde	A.S.T.M.
		Approx. satn. water content	Approx. satn. water content	24-hr. water absorption
38-12 <sup>a</sup>	0.0	..	29.8	12.9
31-118	6.9	34.7	24.1	..
38-8	10.2	35.1	24.6	6.6
38-30	13.9	27.3	22.1	6.2
38-14	16.6	25.0	21.2	5.8
38-18	17.3	23.0	20.0	4.5
38-22	17.4	24.9	21.1	5.6
31-187	18.0	21.6	20.6	5.7
31-150	18.5	20.5	19.1	4.4
38-26	19.6	21.1	19.1	4.1
31-142	21.0	19.3	18.6	4.2
31-145	23.4	20.0	17.7	3.9
38-6	23.6	17.7	17.3	4.1

<sup>a</sup> Control: casein dissolved in alkali and treated with palmitic acid instead of palmitoyl chloride as in the modification procedure (6), then precipitated, hardened with formaldehyde, washed, dried, and extracted with ether.

appreciably from that of the original powder. The temperatures used in the flow measurements were slightly different from those specified, and it was necessary to use higher pressures to obtain adequate flow. These deviations from the standard procedure were necessitated primarily by the presence of water as plasticizer in the molding powders. Every effort was made to maintain the water content of the tablets at the desired levels. Especially with unmodified casein was this factor of paramount importance, since slight differences in water content affected plastic flow markedly.

Table I illustrates the progressive reduction in water absorption with increasing acylation of casein by palmitoyl groups. Hardening with formaldehyde, which is effective in diminishing the water absorption of unmodified casein or of palmitoyl caseins substituted to a low degree, has comparatively little effect on the water uptake of highly acylated palmitoyl caseins. Analysis of the hardened products for formaldehyde showed that the formaldehyde binding capacity of casein had been decreased materially by acylation (6). As expected, curtailment in the affinity of these highly acylated derivatives for water was substantial, but not so pronounced as had been thought possible from the results previously reported for acetylation, propionylation, and butyrylation in nonaqueous media (7).

TABLE II. WATER ABSORPTION OF ACYLATED CASEINS HARDENED WITH FORMALDEHYDE

Sample No.	Acyl Group	Acyl Content, %	Water Absorption of Disks, %	
			Approx. satn. water content	A.S.T.M. 24-hr. water absorption
38-12 <sup>a</sup>	.....	0.0	29.8	12.9
38-93	Caprylyl	21.5	15.3	2.5
38-87	Pelargonyl	17.1	16.8	..
38-98	Capryl	20.9	15.1	2.5
38-64	Lauroyl	23.7	14.4	..
38-76	Myristoyl	26.0	14.7	2.7
38-61	Palmitoyl	22.5	18.0	4.5
31-137	Oleoyl	23.8	16.3	3.6
31-152	Stearoyl	23.2	18.8	..

<sup>a</sup> Control: See footnote, Table I.

Table II lists the results of further experiments to determine the influence of increasing size of substituent group on the water absorption of casein acylated in aqueous media and then hardened with formaldehyde. When approximately the same bulk of acyl groups (about 20% acyl) was present in the derivatives, variation in the size of acyl radical from C<sub>8</sub> to C<sub>18</sub> did not affect the water uptake greatly. The more readily available higher fatty acid chlorides were therefore used for the preparation of most of the modified caseins investigated.

Results of water absorption tests on palmitoyl derivatives of different proteins hardened with formaldehyde are recorded in Table III. Apparently the modification reaction is a general one

in so far as it has been applied, and effects a curtailment of the affinity of the unmodified proteins for water. The reaction with wheat gluten was not very satisfactory, as may be seen from the low degree of acylation attained; this may explain the relatively high water absorption of the derivative. The test results for palmitoyl zein are noteworthy, inasmuch as they are the lowest figures for a water-plasticized protein derivative yet obtained in our several series of modified proteins.

TABLE III. WATER ABSORPTION OF PALMITOYL PROTEINS HARDENED WITH FORMALDEHYDE

Sample No.	Protein and Source	Palmitoyl Content, %	Water Absorption of Disks, %	
			Approx. satn. water content	A.S.T.M. 24-hr. water absorption
31-173	Egg albumen (sol., Eimer and Amend)	17.1	15.9	3.4
38-65 <sup>a</sup>	Zein (Corn Products Refining)	22.9	11.1 <sup>b</sup>	2.1
38-51	Soybean (alpha, Glidden)	19.5	18.9	..
38-53	Soybean (ortho, Drackett)	20.7	20.4	6.1
38-55	Peanut <sup>c</sup>	21.7	20.0	6.5
38-57	Cottonseed <sup>c</sup>	22.1	20.0	5.6
38-59	Wheat gluten (Pfanzstiehl)	11.2	38.9	28.9

<sup>a</sup> This derivative was not treated with formaldehyde; such hardening had no appreciable effect on the physical properties of palmitoyl zein.

<sup>b</sup> The corresponding figure for unmodified zein is 47.4, and for zein hardened with formaldehyde, 21.2%. Attempts to obtain a comparable figure for unmodified casein were unsuccessful because the molded disks, on immersion in water, disintegrated before approximate saturation was reached.

<sup>c</sup> Furnished by the Oil, Fat, and Protein Division of the Southern Regional Research Laboratory.

Data on the tensile and flexural strengths of a number of palmitoyl caseins are shown in Table IV. It is clear that acylation sufficient to produce substantial reduction in water absorption is accompanied by a considerable drop in tensile and flexural strengths. The loss in strength appears to be progressive with increasing acylation and perhaps related to the pH of the modification procedure. At approximately the same level of acylation the products prepared under more alkaline conditions tend to be weaker. Regardless of the degree of acylation the observed tensile and flexural strengths of the derivatives hardened with formaldehyde are higher than those of the corresponding unhardened materials. Thus, it must be concluded from the data at hand that, of our acylated casein products, the ones of most promise from a practical standpoint may be those acylated to a moderate extent and then hardened with formaldehyde. Such derivatives prepared at pH 9 have reasonably good strengths and, although not so resistant to water as some of the other modified caseins, have water absorptions lower than that of commercial casein plastics (7 to 14% in 24 hours as determined by the A.S.T.M. method). Test data for derivatives of this type are summarized in Table V. Variations in properties may be attributed to difficulties in controlling the modification procedure rather than to differences in the nature of acyl groups substituted.

The flexural strengths shown in Tables IV and V are of interest for comparative purposes, but since they are in most cases lower than the corresponding tensile strengths they are doubtless somewhat low in an absolute sense. This is probably due to the difficulty of properly conditioning the test bars. Although the conditioning period of a week was found sufficiently long to permit approximate equilibration of the moisture in the tensile test specimens with that of the conditioning atmosphere, it was recognized that such equilibration could not be attained with the thick flexural test bars in the same length of time; nor was equilibration to be expected in any reasonably longer time. For convenience, therefore, the conditioning period was kept uniformly at one week.

Comparative figures for the plastic flow of palmitoyl caseins under a given set of conditions are listed in Table VI. In the

main, increasing acylation results in higher plastic flow. Hardening with formaldehyde clearly decreases plastic flow. Nevertheless, hardened, highly acylated caseins flow much better than formaldehyde-hardened casein, almost as well as unmodified casein in the presence of 12% water, and better than unmodified casein in the presence of 8.5% water. That these derivatives flow well in the presence of relatively small amounts of water is of importance because, as a consequence, small plastic pieces with considerable dimensional stability can be molded from them. It is true, however, that the excellent plastic flow of unmodified casein containing relatively large amounts of water (15% or more) cannot be equaled by the modified proteins.

## DISCUSSION

Acylation of casein with higher fatty acid radicals results in products with decreased affinity for water and with improved plastic flow properties. With 6 to 12% water as plasticizer these products can be molded into small transparent objects which do not warp or check; nor do they shrink excessively because of water exchange. These advantages become more pronounced with increasing acylation. At the same time increasing acylation adversely affects the strength of the molded pieces, so that some sacrifice of these advantages may be necessary in the interest of strength. Similarly, hardening the acylated products with formaldehyde before molding, a step which does not materially reduce water absorption and which adversely affects plastic flow, may be necessary in the interest of strength.

Although most of the derivatives studied were palmitoyl proteins, oleoyl chloride as the modifying reagent appears to have at least one advantage—namely, that the fatty acid resulting from hydrolysis is a liquid and can more readily be removed by extraction. A further possible advantage—that the introduction of a number of unsaturated linkages in the form of oleoyl radicals might result in the formation of desirable cross linkages during the molding operations—could not be demonstrated in these experiments.

Of the products obtained by the modification of proteins other than casein, palmitoyl zein merits further mention. As previously noted, this product had an outstandingly low water absorption. Furthermore, hardening with formaldehyde was without effect on either the water absorption or tensile strength of the test specimens, and this step can be omitted in the preparation of palmitoyl zein. The tensile strength of this material is about 5000 pounds per square inch, and it has comparatively good flow properties.

In general, the derivatives discussed above are superior to the acylated caseins previously described (7) even though the more extensive reduction in water uptake anticipated from the introduction of higher fatty acid radicals was not fully attained. The plastic flow of the newer molding powders is better, and they may be compression-molded in positive-type molds into finished articles which are considerably stronger and less brittle than our earlier products. However, the plastic flow of the powders at 130° C., the maximum temperature which can safely be used for these materials, is not equal to that of some commercial thermoplastic resins at the higher temperatures ordinarily used for the latter. It remains to be seen whether further improvements can be effected by plasticizers other than water.

## CONCLUSIONS

The modification of casein by higher fatty acid residues yields materials which in certain respects are superior to the unmodified protein as plastic molding powders. Progressively higher acylation affects the plastic properties of casein as follows: water absorption is reduced, plastic flow in the presence of 6 to 12% water as plasticizer is improved, and tensile and flexural strengths are decreased. It is concluded that a moderate degree of acylation followed by a short treatment with formaldehyde results in the

TABLE IV. EFFECTS OF pH OF ACYLATION AND HARDENING WITH FORMALDEHYDE ON TENSILE AND FLEXURAL STRENGTHS OF PALMITOYL CASEIN PLASTICS

Sample No.	pH of Acylation	Palmitoyl Content, %	Tensile Strength, Lb./Sq. In.		Flexural Strength, Lb./Sq. In.	
			Un-hardened	Hardened	Un-hardened	Hardened
38-12 <sup>a</sup>	(12)	0.0		10,000		12,500
38-30	8	13.9	4700	5,600	3900	4,300
38-14	9	16.6	3500	5,400	3200	4,700
38-22	10	17.4	2300	4,300	2400	4,300
38-26	11	19.6	2100	3,400	1900	2,700
38-18	12	17.3	2200	2,700	1600	2,600

<sup>a</sup> Control: See footnote, Table I.

TABLE V. PROPERTIES OF ACYLATED CASEINS PREPARED AT pH 9 AND HARDENED WITH FORMALDEHYDE

Sample No.	Acyl Group	Acyl Content, %	Approx. Satn. Water Content, %	A.S.T.M. 24-Hr. Water Absorption, %	Tensile Strength, Lb./Sq. In.	Flexural Strength, Lb./Sq. In.
31-147	Palmitoyl	17.2	21.5	5.2	6200	5600
31-187	Palmitoyl	18.0	20.6	5.7	5900	5500
38-14	Palmitoyl	16.6	21.2	5.8	5400	4700
31-169	Oleoyl	22.2	17.9	4.2	4900	3700
31-181	Oleoyl	15.0	21.3	6.1	7400	7600
38-39	Oleoyl	20.7	20.0	5.3	5700	
38-83	Lauroyl	13.5	20.7	5.7	5800	3600

TABLE VI. PLASTIC FLOW OF PALMITOYL CASEINS

Sample No.	Palmitoyl Content, %	Inches of Flow in 2 Min. at 131° C., 3000 Lb./Sq. In. <sup>a,b</sup>			
		Tablets (H <sub>2</sub> O content, 12%)		Tablets (H <sub>2</sub> O content, 8.5%)	
		Unhardened	Hardened	Unhardened	Hardened
88 <sup>c</sup>		0.99		0.30	
38-12 <sup>d</sup>	0.0		0.16 <sup>e</sup>		0.11 <sup>e</sup>
38-30	13.9	0.77	0.47	0.61	0.25
38-14	16.6	1.06	0.50	0.62	0.27
38-18	17.3	1.10	0.61	0.68	0.32
38-26	19.6	1.26	0.88	0.85	0.45
31-145	23.4	1.33	0.92	0.91	0.53
38-6	23.6	1.28	0.64	0.98	0.43

<sup>a</sup> Similar sets of data were obtained at 131° C. and 2500 pounds/square inch, 122° C. and 3000 pounds, and 122° C. and 2500 pounds as well as a few figures at 116° C. at various pressures. These results are not tabulated, since they led to the same conclusions presented in the text.

<sup>b</sup> Figures listed are averages of two or, in most cases, three close results.

<sup>c</sup> Unmodified casein, extracted with ether.

<sup>d</sup> Control: See footnote, Table I.

<sup>e</sup> This value is of doubtful significance, since true plastic flow was not observed in the extruded rods; the extruded portions appeared to consist of compressed powder.

best over-all combination of properties. In the case of palmitoyl zein hardening with formaldehyde is unnecessary, and the product has an unusually low water absorption. Test pieces molded from moderately acylated casein are relatively stable in dimensions, have A.S.T.M. water absorptions of 5 to 6% in 24 hours, tensile strengths of 5000–6000 pounds per square inch, and flexural strengths of the same order of magnitude.

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